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ISOZYME ANALYSIS IN SIX POPULATIONS OF *PEDIOBIUS FOVEOLATUS* (CRAWFORD) (HYMENOPTERA: EULOPHIDAE)

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Abstract.—Sixteen enzyme loci were used to determine the degree of genetic variability and taxonomic status of *Pediobius foveolatus* (Crawford) (a hymenopterous parasite of *Epilachna* spp.) from Japan, Korea, China, Hong Kong, Guam, and India. Two loci, ACON-1 and MDH-1 were found to be polymorphic. However, no intra-population variation was found for any of the 16 loci studied. Based on these isozyme analyses, three biotypes were recognized in *P. foveolatus*, namely, the China biotype with its unique ACON-1 genotype, the Northern biotype with MDH-1^s allele, and the Southern biotype with MDH-1^f allele.

Key Words: allelic repression, biotype, electrophoresis, *Epilachna*

Pediobius foveolatus (Crawford) is a gregarious parasite of larvae of *Epilachna* spp. (Coleoptera: Coccinellidae). It is found naturally in widespread areas of the Asiatic, Australasian, and African regions (Kerrich 1973). In recent years it has been reported in Japan (Tachikawa 1976), Sumatra (Abbas and Nakamura 1985), and China (Schaefer et al. 1986). Recent collections have recovered it in South Korea and in Hong Kong (Schaefer, unpubl. data).

In the early 1950's, under the name *Pleurotropis epilachnae* Rohwer, this parasite was intentionally introduced into Guam from the Philippines to combat the introduced *Epilachna philippinensis* Dieke (Peterson 1955). It was introduced into North America from Bangalore, India, for the control of Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant, in 1966 (Angalet et al. 1968). It failed to overwinter in the U.S. but has repeatedly shown promise as it readily attacks MBB larvae during the season of its release. In the belief that a race from temperate areas might be capable of surviving winters in North America, one of us (P.W.S.) obtained *P. foveolatus* (hereafter referred to as *Pediobius*) from Honshu, Japan, and first released it in North America in 1980 but this race also did not permanently establish (Schaefer et al. 1983). We have concluded that *Pediobius* probably requires an alternate host *Epilachna* sp. which overwinters as a larva or pupa. In parts of Asia there are some species with this trait. The only three species in the Epilachninae in the eastern U.S. all overwinter as adults.

At the time of the release of the Japanese *Pediobius*, we attempted to distinguish this race from the Indian one which, at the time, was being released in widespread projects in several mid-Atlantic states (thus necessitating our releases in the Mississippi River valley). Being unable to distinguish these two races morphologically, we turned to electrophoretic analysis to provide a marker

Table 1. History of cultures of *Pediobius foveolatus* at BIRL.

Designation	Locality	Date of Receipt	Starter Sample Size	No. Generation in Laboratory
Japan (A)	Kurashiki, Honshu	8/15/79	2878 M&F[a]	131
Japan (B)	Yashiro, Honshu	8/27/80	1497 M&F	108
Korea	Seoul	10/04/82	259 M&F	63
China	Beijing & Taiyuen	11/07/84	3M, 79F	23
Hong Kong	Fanling, New Territory	8/05/82	2271 M&F	44
	Fanling, New Territory	8/06/83	352 M&F	
Guam	Mangilao	6/24/85	18M, 86F	11
India (A)	Devanahalli, Bangalore	5/24/72[b]	398 M&F	286[d]
	Avali, Bangalore	7/5–8/21/73[c]	2814 M&F	
India (B)	Re-colonized subculture from (A)	(Same as above)		286[d]

[a] M = males, F = females.
[b] Only one of four 1972 shipments (with about 30 females) contributed to the laboratory culture.
[c] Four shipments including 1596 females.
[d] Approximate generation number based on calculations.

which might identify the Japanese population. As more races became available, we extended this study into an assessment of the genetic heterozygosity of *Pediobius* from widely scattered geographical locations (Japan, Korea, China, Hong Kong, India, and Guam) as well as some of the inter-cross races maintained in culture at Beneficial Insects Research Laboratory (BIRL), Newark, Delaware. We report on the electrophoretic means of distinguishing some populations from others and on the overall genetic heterozygosity of this species based on reared material which originated from the locations given.

Materials and Methods

Living material was obtained from cultures being reared at BIRL. The origin and brief history of each culture, date(s) of importation, starter sample size, and the generation number (or approximation) of each culture are presented in Table 1. The ultimate rearing procedure was to maintain each culture in unwaxed paper cups (112 mm ID at top, 55 mm deep, capacity 470 ml) with clear plastic lids. Sting units were set up approximately every two to three weeks by aspirating ca. 20–30 adults (ca. equal sex ratio) from old cups and expelling them into new cups which contained 25 fourth instar MBB larvae. These sting units sat for ca. 6 h, after which all *Pediobius* were collected by aspiration and destroyed. Care was taken never to return specimens into cups once they were removed or escaped. This was done as a precaution to prevent any genetic mixing of indistinguishable wasp strains. The host larvae were then transferred to reusable rigid polyethylene cylindrical cages (158 mm ID, 120 mm deep) with three fine mesh screen portals (5 cm dia.) on the sides for air circulation and inverted glass pie plates for covers. Food was provided as a bouquet of Tendergreen snapbean plants in a glass vial of water with cotton plugging firmly holding the plant stems. Food was replaced during the first week if needed. During the second week host larvae were transferred into new paper cups to await adult emergence. Just prior to expected emergence (two to three weeks depending on the seasons since ambient indoor temperature and humidity was not regulated), the inner surface of the lid was smeared with honey as the only food source. Water was not usually provided but on occasion if drying was evident, water was injected through the cup wall using a hypodermic needle.

Material used in the electrophoretic analysis was taken from the cup after a subsam-

Table 2. Gel and electrode buffer systems.

Designation	Description	Source
MC	N-(3-Aminopropyl)-morpholine-citrate (pH 6.0)	Clayton and Tretiak (1972)
PK	Discontinuous Tris-citrate (Electrode: pH 8.2; Gel: pH 8.7)	Poulik (1957)
TC	Tris-citrate (pH 7.0)	Siciliano and Shaw (1976)
TVB	Tris-versene-borate (pH 8.0)	Siciliano and Shaw (1976)

ple was removed to begin the next generation. Specimens in their intact paper cups were shipped or hand-carried to Beneficial Insects Laboratory (BIL), Beltsville, Maryland, and samples were then frozen and stored at −65°C until electrophoresis.

Electrophoretic Methods

Electrophoresis was performed on horizontal starch gels for 4 h using 6% Electrostarch, 6% Sigma starch, 5% sucrose, 10 mg NADP, and 400 ml gel buffer (see Table 2). The 12 enzyme systems examined are listed in Table 3, each followed by the buffer systems used. At least six female and six male wasps per enzyme per culture were electrophoresed. Sample sizes were increased to over 20 for loci that exhibited polymorphisms. At least 18 female progeny were used in the controlled progeny analyses of genotypes at ACON-1 and MDH-1 loci.

We assumed that discrete zones of enzyme activity were controlled by single loci coding for specific products. The genetic basis of observed isozyme variations (see ACON and MDH below) were confirmed by progeny analyses. Loci coding for the same enzyme are numbered sequentially from the most anodal to the most cathodal regions of activity. The allelic proteins are designated alphabetically, with "A" the fastest running allele. The allelic designations in ACON-1 and MDH-1 were inferred from progeny analyses and were labelled as "F" or "S."

Results and Discussion

In addition to the enzymes shown in Table 4, we also examined acid phosphatase, aldehyde oxidase, esterase, galactose-6-phosphate dehydrogenase, glutamate dehydrogenase, leucine aminopeptidase, and superoxide dismutase. However, these enzymes had either very weak or streaky bands

Table 3. Enzymes analyzed in *P. foveolatus*, with buffer conditions employed.

EC* no.	Enzyme	Buffer System
4.2.1.3	aconitase (ACON)	MC
1.1.1.49	glucose-6-phosphate dehydrogenase (G6PD)	MC
2.6.1.1	glutamate-oxaloacetate transaminase (GOT)	MC
1.1.1.8	alpha-glycerophosphate dehydrogenase (GPDH)	TC, TVB
2.7.1.1	hexokinase (HK)	PK, TVB
1.1.1.42	isocitrate dehydrogenase (IDH)	TC
1.1.1.27	lactate dehydrogenase (LDH)	TC
1.1.1.37	malate dehydrogenase (MDH)	MC, TC
1.1.1.40	malic enzyme (ME)	TC
1.1.1.44	6-phosphogluconate dehydrogenase (6PGD)	MC
5.3.3.9	phosphoglucose isomerase (PGI)	MC, TC
2.7.5.1	phosphoglucomutase (PGM)	MC, PK

* Enzyme Commission.

Table 4. Isozyme phenotypes of six *Pediobius foveolatus* populations.

Enzyme locus	Population**					
	Japan	Korea	China	Hong Kong	Guam	India
ACON-1	FF	FF	SS	FF	FF	FF
ACON-2	AA	AA	AA	AA	AA	AA
GOT	AA	AA	AA	AA	AA	AA
G6PDH	AA	AA	AA	AA	AA	AA
GPDH-1	AA	AA	AA	AA	AA	AA
GPDH-2	AA	AA	AA	AA	AA	AA
HK-1	AA	AA	AA	AA	AA	AA
HK-2	AA	AA	AA	AA	AA	AA
IDH	AA	AA	AA	AA	AA	AA
LDH	AA	AA	AA	AA	AA	AA
MDH-1	SS	SS	FF	FF	SS	FF
MDH-2	AA	AA	AA	AA	AA	AA
ME	AA	AA	AA	AA	AA	AA
PGM	AA	AA	AA	AA	AA	AA
PGI	AA	AA	AA	AA	AA	AA
6PGD	AA	AA	AA	AA	AA	AA

** Japan (A) and Japan (B) were combined as there were no differences between them. India (A) and India (B) were also combined for the same reason.

that could not be clearly scored. Therefore, only data on 12 enzyme systems were used in this analysis (Table 4).

All the enzymes in this study migrated anodally with the buffer conditions employed except ACON-2, MDH-2 and 6-PGD which migrated cathodally (MDH-1 and MDH-2 also migrated cathodally in the MC buffer). Only four enzyme systems had more than two loci and with the exception of ACON-1 and MDH-1, all other loci were monomorphic (Table 4). Progeny analyses also revealed that ACON is monomeric and MDH is dimeric in *Pediobius*.

Although the five populations we studied were identical at 14 loci, there are some differences at the other two loci. As shown in Table 4, two alleles were found at both ACON-1 and MDH-1 with each population fixed for one of the two alleles at each locus. The China population was unique in being fixed for the slow allele at ACON-1 while the others were fixed for the fast allele at this locus. Guam, Japan, and Korea populations were fixed for the MDH-1^s allele while samples from China, Hong Kong, and India were fixed for the MDH-1^f allele.

Although Hung et al. (1986) reported high levels of genetic heterozygosity in the hyperparasitic wasp, *Mesochorus nigripes*, very low levels of electrophoretic variation have been found in most hymenopteran species. Some species even lack variation altogether (Wagner and Briscoe 1983). The genetic homogeneity found within each population at these 16 loci possible is not the result of founders effects, because even the smallest sample (China culture) originated from 79 female wasps collected from two widely separated localities. This lack of enzyme variation might be due to inadvertent selection during long periods of laboratory rearing as in the case of the screwworm fly (Bush et al. 1976). Since the starter materials were not analyzed electrophoretically, the validity of this assumption cannot be ascertained.

Pleurotropis epilachnae, described as a separate species from India by Rohwer, was synonymized under *Pediobius foveolatus* (Crawford) by Kerrich (1973), because the differences in size and color did not hold up in specimens other than the type series. Therefore, our samples were all identified as *P. foveolatus* and no morphological differences were found among them (M. E. Schauff, pers. comm.).

According to Peterson (1955), *Pleurotropis epilachnae* Rohwer (= *P. foveolatus*) was successfully introduced into Guam from the Philippines during 1954 to control the phytophagous ladybeetle, *Epilachna philippinensis* Dieke. The BIRL quarantine records also show that the only recorded shipments to Guam occurred in 1974 (three shipments) and 1975 (three shipments) and are recorded as origin "India." The Japan culture was first obtained in August 1979, and has never knowingly been imported into Guam. Therefore, it is rather puzzling that the Guam population was the same as the Japan and Korea populations in having only

the slow allele at MDH-1. Over 30 female wasps from the Guam culture analyzed all had only the slow allele and it is not likely that this culture was contaminated. We cannot explain the apparent contradiction between our findings and the implications based on historical records.

Our cultures of these six populations crossed successfully in both directions under laboratory conditions. Peng (1988) also reported that there was no reproductive isolation between his Beijing *Pediobius* and those from Hong Kong, India, Japan, and Korea that he received from PWS. However, he reported that "malic dehydrogenase (sic)" was one of three enzymes that were monomorphic in the five cultures he studied. This is different from our results as shown in Table 4. It is possible that the lack of variability in his malate dehydrogenase study is due to the poor resolution of the buffer system and supporting medium he used (see Hung and Vinson 1977).

Four patterns of allelic repression have been reported in interspecific as well as intertribal hybrids (Avise and Duvall 1977, Hung and Vinson 1977): (a) repression of paternal protein synthesis; (b) repression of maternal protein synthesis; (c) repression of both maternal and paternal protein synthesis; and (d) caste specificity of differential parental protein synthesis in social insects. Although the slow allele at the ACON-1 locus was found only in the China population, our controlled progeny analyses of genotypes at ACON-1 locus did not reveal any allelic repression as is frequently observed in interspecific hybridization (e.g. Hung and Vinson 1977, Hung 1985, Hung and Norden 1987). Both maternal and paternal genes were fully expressed in F_1 and F_2 progeny in our two-way crosses. Therefore, we do not recognize the China population as a separate species. Differences in the percentage of ovipositing females, average fecundity and longevity of females, and number of hosts parasitized were found between the Indian and the Japan "races" of *P. foveolatus* (L. Nong, pers. comm.). However, we believe these biological differences only signify that they are two biotypes. Based on these isozyme analyses, we concluded that there are three biotypes in *P. foveolatus*: I. China biotype with its unique ACON-1 genotype; II. Northern biotype (Japan and Korea) with MDH-1^s allele; and III. Southern biotype (India and Hong Kong) with MDH-1^f allele. The introduced population from Guam apparently belongs to the northern biotype contrary to the expectations based on the historical shipment records.

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